

## Chronic Conjunctivitis Caused by Oral Anaerobes and Effectively Treated with Systemic Metronidazole plus Amoxicillin

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**In this study, we report on a case of refractory, unilateral anaerobic conjunctivitis. The predominant anaerobic flora consisted of *Prevotella intermedia* (formerly *Bacteroides intermedius*) and *Peptostreptococcus micros*. By using the technique of restriction endonuclease fingerprinting of genomic DNA, it was shown that the *P. intermedia* likely originated from the oral cavity. Topically applied antibiotics had failed to suppress the infection in the past. Successful treatment was achieved after systemic administration of metronidazole plus amoxicillin.**

Obligately anaerobic bacteria occur in the conjunctival sac in most individuals and are probably part of the normal eye flora. The predominant anaerobic species found in the healthy conjunctival sac is *Propionibacterium acnes*, whereas peptostreptococci, lactobacilli, *Bacteroides* spp., and *Clostridium* spp. can be isolated infrequently (8, 9, 11).

Anaerobic bacteria have been implicated in the etiology of ophthalmic infections; however, their role in this type of infection is poorly understood. Whether these anaerobes are true residents of the conjunctival sac or transient inhabitants is still uncertain. If they are transient, their natural habitat is not known.

In this study, we report a patient with a chronic, refractory purulent conjunctivitis associated with large numbers of anaerobic bacteria. In addition, we show that these microorganisms likely originated from the oral cavity. The condition responded to only systemic antibiotic therapy.

### MATERIALS AND METHODS

**Patient description and treatment history.** The patient was a 33-year-old male who was referred to a periodontal clinic for severe inflammation of the gums. Clinical examination revealed a generalized form of periodontitis, including a purulent inflammation of deep periodontal pockets and severe loss of alveolar bone in most parts of the jaws. The periodontist also noticed a severe dacryopyorrhea of the left eye. According to the patient, he had suffered from this disorder for the last 3.5 years; 1.5 years before the condition developed, he had suffered from ocular trauma. The patient did not use contact lenses. He consulted an ophthalmologist, who diagnosed conjunctivitis and prescribed topical tetracycline. One year later, the disease was diagnosed as a keratoconjunctivitis and was treated topically with the combination of oxytetracycline, polymyxin B, and hydrocortisone (Terra-Cortril; Pfizer). A second ophthalmologist made the diagnosis of follicular conjunctivitis, and the patient was treated initially with tetryzoline, subsequently with topical tobramycin, and later with the combination of prednisolone and neomycin (Predmycin-P Liquifilm; Allergan). None of these treatments resulted in a cure. At the time of referral to

the periodontal clinic, the patient was being treated with a combination of several homeopathic compounds.

For the purpose of this study, a third ophthalmologist, from the Department of Ophthalmology of the Vrije Universiteit Amsterdam, was consulted for diagnosis and clinical examination. The diagnosis of unilateral conjunctivitis was confirmed. No other disorders, such as dacryocystitis, canaliculitis, or sinusitis, were observed.

**Sampling and cultivation.** Oral samples for microbiological investigation were taken from the tonsils and the dorsum of the tongue with swabs. The deepest periodontal pockets with bleeding on probing and the conjunctival sac were sampled with sterile paper points. Samples were immediately transported to the laboratory in a reduced transport medium (RTF) (13). After the samples were dispersed on a vortex mixer, 10-fold serial dilutions were prepared in RTF. Aliquots of 0.1 ml were inoculated onto 5% horse blood agar plates (Oxoid no. 2; Oxoid Ltd., Basingstoke, England) supplemented with hemin (5 mg/liter) and menadione (1 mg/liter). The plates were incubated anaerobically in an atmosphere of 80% N<sub>2</sub>, 10% CO<sub>2</sub>, and 10% H<sub>2</sub> at 37°C for 7 days or aerobically for 5 days. After pure cultures of the isolates were established, the following characteristics of the isolates were determined: colony morphology, Gram stain, oxygen tolerance, fermentation of sugars, production of indole from tryptophan, and the production of specific enzymes with the API ZYM system (API Laboratories). Isolates were identified to the species level on the basis of the criteria presented by Johnson and Holdeman (5), van Winkelhoff et al. (15, 17), and Holdeman et al. (4).

Testing of the susceptibility of the anaerobic isolates to neomycin, tetracycline, tobramycin, amoxicillin, and metronidazole was performed by the agar dilution reference method (10).

**Restriction endonuclease analysis of DNA.** To establish a possible clonal origin for the bacteria isolated from the oral cavity and the conjunctival sac, DNA from the bacterial isolates was subjected to restriction endonuclease analysis by the method described by Loos et al. (7). In short, DNA from a sufficient number of bacterial cells was obtained after lysis of late-log-phase cells and purified by repeated phenol-chloroform extraction and by treatment with RNase. The DNA was digested with endonuclease *Pst*I. After digestion, the DNA fragments were separated by electrophoresis in an

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TABLE 1. Occurrence of anaerobic bacteria in the oral cavity and in pus from the conjunctival sac

Species	% Identified flora isolates <sup>a</sup> from:			
	Subgingival area	Tongue	Tonsil	Eye
<i>Porphyromonas gingivalis</i>	58	0	0	0
<i>Prevotella intermedia</i>	0.2	2	0.5	74
<i>Peptostreptococcus micros</i>	4.1	1	1	15

<sup>a</sup> Percentage of anaerobically cultivable flora identified as the indicated species.

agarose gel. The DNA bands were visualized by staining the gel with ethidium bromide. Finally, the gel was photographed with a Polaroid camera.

### RESULTS

The aerobic culture of the pus from the conjunctival sac showed only a few colonies of gram-positive cocci, which were not considered significant. In contrast, the anaerobic culture revealed heavy growth of two main species, which were identified as the black-pigmented *Prevotella intermedia* (formerly *Bacteroides intermedius*) and *Peptostreptococcus micros* (Table 1). The additional growth consisted of non-sporeforming gram-positive and gram-negative bacilli. *P. intermedia* and *P. micros* were also isolated from the dorsum of the tongue, the tonsillar area, and the periodontal pockets. In addition, the periodontal pockets contained large numbers of bacterial species, with *Porphyromonas gingivalis* (formerly *Bacteroides gingivalis*) predominating (Table 1). The restriction endonuclease digestion profiles of the *P. intermedia* DNAs from the conjunctival sac, the tonsillar area, and the dorsum of the tongue appeared to be identical but were different from that of the DNA of the *P. intermedia* isolates from the periodontal pockets (Fig. 1).

Susceptibility testing of the *P. intermedia* and the *P. micros* strains revealed resistance to tobramycin for both



FIG. 1. Duplicate DNA fingerprints (*Pst*I digests) of eight *P. intermedia* strains. Identical profiles are seen for isolates from the conjunctival sac (lanes 4 and 5), the tongue (lanes 6 and 7), and the tonsillar area (lanes 8 and 9). Different profiles are seen for the *P. intermedia* strains from the periodontal pockets (lanes 2 and 3). Lanes 1 and 10, Lambda phage DNA digests.

strains (MICs, >100 and 50  $\mu$ g/ml, respectively). Both strains were susceptible to tetracycline (MICs, 0.625 and 0.3  $\mu$ g/ml, respectively), amoxicillin (MICs, 0.5 and 0.25  $\mu$ g/ml, respectively), and metronidazole (MICs, 1.0 and 1.0  $\mu$ g/ml, respectively) and moderately susceptible to neomycin (MICs, 60 and 50  $\mu$ g/ml, respectively).

On the basis of the periodontal condition and the results of the anaerobic cultures from the conjunctival sac, the patient was given systemic antibiotic therapy which consisted of metronidazole (750 mg/day) and amoxicillin (1,125 mg/day), both for a period of 7 days. This therapy resulted in a rapid improvement of the inflamed conjunctiva. After 6 days of therapy, swelling, redness, and suppuration had disappeared. Repeated cultivation during the following 4 months revealed a total absence of both *P. intermedia* and *P. micros*, in association with a normal appearance of the conjunctiva.

In addition, as a result of mechanical treatment and the antibiotic therapy, the condition of the gingiva significantly improved. Suppuration had disappeared, and pocket probing depths and clinical attachment levels significantly improved.

### DISCUSSION

Ophthalmic infections are commonly caused by aerobic and facultatively anaerobic bacteria. However, anaerobic eye infections also occur and possibly more often than is generally thought, since anaerobic bacteriology is not usually performed for superficial eye infections. Previous studies have pointed to the possible role of *Peptostreptococcus* spp. in conjunctivitis (1, 9). So far, black-pigmented gram-negative anaerobes such as *P. intermedia* have not been implicated in chronic conjunctivitis. Experimental infections in laboratory animals involving *Peptococcus* spp. and *P. intermedia* have shown that synergy between these microorganisms is possible (6).

On the basis of the microbiological data obtained for the patient in this study, the possibility that the infection was caused initially by microorganisms other than *P. intermedia* and *P. micros* cannot be excluded. Striking features in this patient were the chronic nature of the infection, the fact that it never spread to the other eye, and the refractory nature of the infection. The ocular trauma, caused by a branch, may have been related to the infection, although this remains uncertain considering the time interval between this event and the clinical manifestation of the infection.

On the basis of the similarity of the DNA digest profiles of the *P. intermedia* isolate from the conjunctival sac and the isolates from the oral cavity, we may plausibly assume that transmission from the oral cavity to the eye had occurred. The phenomenon of transmission of oral bacteria to the eye may occur much more often than previously thought, considering the bacterial species reported for eye infections. These species include facultatively anaerobic streptococci, fusobacteria, *Veillonella* spp., lactobacilli, *Actinomyces* spp., and anaerobic streptococci (12). These species are part of the normal oral microbiota, being present on mucous membranes, in dental plaque, and in saliva.

The technique of restriction endonuclease fingerprinting of genomic DNA has been described as a useful tool in epidemiology and transmission studies for several bacterial species (2, 7). The usefulness of this technique for *P. intermedia* has been confirmed recently (14). In that study, more than one clonal type of *P. intermedia* was found in the oral cavity, as we have shown in the present study.

The choice of systemic treatment with metronidazole and amoxicillin was based on the observations that topical anti-

biotic treatment had repeatedly failed. The choice of metronidazole and amoxicillin was based on the susceptibilities of the anaerobic isolates and on our experience with the efficacy of these antibiotics for certain periodontal infections (3, 16). The present report shows that this combination of antibiotics is also very effective against mixed anaerobic infections. It is not clear why the topically applied antibiotics failed to suppress the pathogenic microfloras, despite the fact that both strains were susceptible to most of the antibiotics used in the past.

We conclude that anaerobic bacteriology can be of importance in ophthalmic infections, especially in refractory cases. In these cases, systemic antibiotic therapy may have advantages over topical treatment. Furthermore, it seems possible that oral bacteria colonize the conjunctival sac and that they are able to cause conjunctivitis.

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